REMARKS

Examination of claims 65-78 is reported in the present Office Action. Claims 65-78 were provisionally rejected, and claims 65-68 and 71-78 were rejected, under the judicially-created Doctrine of Obviousness-Type Double Patenting. Claims 65-72, 74, 77, and 78 were rejected under 35 U.S.C. § 102(e), and claims 65, 72, and 73 were rejected under 35 U.S.C. § 103(a). Claims 75 and 76 were objected to as depending from a rejected base claim. Each of the rejections and the objection are addressed as follows.

First, applicants note that, in reply to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures that was enclosed with the Office Action, applicants enclose herewith a Sequence Listing in compliance with 37 C.F.R. §§ 1.821-1.825.

Obviousness-type Double Patenting Rejections

Claims 65-72, 74, 77, and 78 were provisionally rejected under the judicially-created Doctrine of Obviousness-type Double Patenting over claims 95, 108-120, 135-175, 191-194, 196-210, 213-247, 264-274, 293-303, 317-327, 349-352, 367-370, 383-385, 389-392, 393-395, and 399-403 of U.S. Serial No. 08/406,030. Claims 73, 75, and 76 were provisionally rejected for Obviousness-type Double Patenting over claims 95, 108-120, 191-194, 196-210, 238-243, 264-271, 293-300, 317-324, 349, 367, 383, 389, and 393-395 of U.S. Serial No. 08/406,030, in view of Capecchi (Science 244:1288-1292,

1989). These provisional rejections should be withdrawn, as U.S. Serial No. 08/406,030 is no longer pending, and the patent into which it issued, 5,733,746, has been withdrawn.

Claims 65-68 and 71-78 were rejected for Obviousness-type Double Patenting over claims 1, 2, 4-10, and 12-24 of U.S. Patent No. 6,063,630. This rejection is being met by the filing of the enclosed terminal disclaimer, which specifies that the term of any patent issuing in the present case will not be longer than the term of the cited patent. This rejection can thus now be withdrawn.

Rejection under 35 U.S.C. § 102(e)

Claims 65-72, 74, 77, and 78 were rejected under § 102(e) as being anticipated by Sherwin et al. (U.S. Patent No. 6,015,708). This rejection is respectfully traversed.

In the presently claimed invention, a regulatory region is introduced into the genome of a primary or secondary cell by homologous recombination, so that the regulatory region alters expression of a gene in the genome of the cell. The present amendment adds the requirements that the cells into which the DNA is introduced be non-immortalized and that cells produced by the claimed methods supply the product of the gene. As is discussed further below, the methods and cells of Sherwin, as well as the concepts of Sherwin, are different from those of the presently claimed invention.

Primary and secondary cells, as used in the present claims, are cells that have been isolated from a vertebrate tissue source or tissue explant, prior to plating or after plating for the first time (primary cells), or such cells at subsequent stages of culturing (secondary

cells) (see, e.g., page 6, line 21 through page 7, line 4 of the specification). These types of cells are thus related to one another by the fact that the primary cells become secondary cells upon further culturing.

As is discussed further below, the use of primary or secondary cells as is specified in the present claims is not described by Sherwin. In particular, the so-called "primary" cells of Sherwin are cells into the genomes of which an amplifiable gene and a regulatory region have been introduced by homologous recombination, and Sherwin's "secondary" cells, rather than being descendants of the initial, "primary" cells, are cells into which DNA that is removed from the "primary" cells is introduced. Thus, the terms "primary" and "secondary" as used by Sherwin describe cells from which DNA has been removed (the "primary" cells) and cells into which this DNA is then introduced (the "secondary" cells).

These very different meanings of the terms "primary" and "secondary" are made clear throughout the text of Sherwin. For example, at column 2, lines 33-37, Sherwin states:

> Expression of mammalian proteins is achieved by homologous recombination, where a DNA sequence is integrated into the genome or large fragment thereof for enhancing the expression of the target gene. The modified sequence may then be transferred to a secondary host for expression. (Emphasis added.)

Further, in a section of the patent that describes the use of yeast cells, Sherwin states that "The YAC will normally be transferred from the original yeast cell to a different yeast host which is convenient for manipulation." (Column 4, lines 28-30.)

The teachings of Sherwin further differ from the present claims in that the only "secondary" cells (i.e., the cells in which expression of DNA obtained from the "primary" cells occurs) specifically mentioned by Sherwin are the following immortalized cell lines:

> Various secondary mammalian expression hosts are available and may be employed. These hosts include CHO cells, particularly DHFR deficient cells, monkey kidney cells, C127 mouse fibroblasts, 3T3 mouse cells, VERO cells, etc. (Column 8, lines 52-55.)

In contrast to these "secondary" cells, the secondary cells of the present claims, as clarified by the amendment made herein, are non-immortalized cells.

Moreover, the "primary" cells of Sherwin are not used to supply a product, as are the primary cells of the present claims. Rather, the "primary" cells of Sherwin are used in homologous recombination methods, and then DNA from these cells is transferred into "secondary" cells for further use, such as in gene product expression.

Thus, because the primary and secondary cells of the present claims differ from those of Sherwin, this rejection can now be withdrawn. Also, for completeness of the record, applicants note that the priority applications of Sherwin add nothing to change the analysis and conclusion provided above.

Rejection under 35 U.S.C. § 103(a)

Claims 65, 72, and 73 were rejected under § 103(a) for obviousness over Sherwin (U.S. Patent No. 6,015,708), in view of Capecchi (Science 244:1288-1292, 1989). This rejection is respectfully traversed.

The Examiner states that Sherwin differs from the claimed invention in not teaching the use of negative and positive selection markers, but that the use of such markers in the methods of Sherwin would have been obvious based on the teachings of Capecchi. In particular, the Examiner states that Capecchi teaches the use of such markers and motivation to use them would have come from Capecchi's teaching that use of these markers can lead to enrichment for desired cells.

As is noted above, Sherwin does not teach key features of applicants' invention: the use of non-immortalized primary and secondary cells to supply a product. Sherwin also does not provide any motivation to use such cells for this purpose, as Sherwin teaches that DNA is to be removed from their so-called "primary" cells and then introduced into so-called "secondary" cells, which are immortalized cells. The use of non-immortalized cells to supply a product, as is required by the present claims, is a concept that Sherwin does not even come close to suggesting. Capecchi does not provide what Sherwin lacks in supporting a rejection of the present claims for obviousness, as Capecchi also does not provide any suggestion to use non-immortalized primary or secondary cells, as defined by applicants, to supply a product. Thus, the teachings of Sherwin, combined with Capecchi's teaching of positive and negative selection markers, do not support a rejection of the claimed invention for obviousness. This rejection can therefore now be withdrawn.

Objection to Claims 75 and 76

Finally, applicants note that claims 75 and 76 were rejected as depending from claim 73, which was rejected, but otherwise were deemed allowable. This objection can now be withdrawn because, in view of the amendments and remarks set forth above, the rejections of claim 73 (as well as the rejections of the claims from which claim 73 depends) should now be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested. Although no charges are believed to be due, if there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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65. (Amended) A method of altering expression of a gene that is present in a non-immortalized primary or secondary cell, said method comprising introducing a DNA construct comprising a regulatory region into the genome of said cell by homologous recombination, wherein said regulatory region is inserted into, or replaces all or a portion of, the regulatory region of said gene, thereby producing a non-immortalized primary or secondary cell in which the expression of said gene is altered so that the product of said gene is supplied.

66. (Amended) The method of claim 65, wherein the non-immortalized primary or secondary cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

67. (Amended) The method of claim 65, wherein the non-immortalized primary or secondary cell is of mammalian origin.

68. (Amended) The method of claim 67, wherein the non-immortalized primary or secondary cell is a human cell.

77. (Amended) A non-immortalized primary or secondary cell produced by the method of claim 65.

78. (Amended) A clonal cell strain of secondary cells produced from a primary or secondary cell [by the method] of claim 77 [65].

65. (Amended) A method of altering expression of a gene that is present in a non-immortalized primary or secondary cell, said method comprising introducing a DNA construct comprising a regulatory region into the genome of said cell by homologous recombination, wherein said regulatory region is inserted into, or replaces all or a portion of, the regulatory region of said gene, thereby producing a non-immortalized primary or secondary cell in which the expression of said gene is altered.

66. (Amended) The method of claim 65, wherein the non-immortalized primary or secondary cell is selected from the group consisting of fibroblasts, keratinocytes, epithelial cells, endothelial cells, glial cells, neural cells, blood cells, muscle cells, hepatocytes, and precursors thereof.

67. (Amended) The method of claim 65, wherein the non-immortalized primary or secondary cell is of mammalian origin.

68. (Amended) The method of claim 67, wherein the non-immortalized primary or secondary cell is a human cell.

69. The method of claim 65, wherein the gene is selected from the group consisting of genes that encode enzymes, cytokines, hormones, antigens, antibodies, clotting factors, regulatory proteins, transcription proteins, and receptors.

70. The method of claim 69, wherein the gene is selected from the group consisting of the human erythropoietin, human growth hormone, human insulin, and human Factor VIII genes.

71. The method of claim 65, wherein expression of the gene is increased.

72. The method of claim 65, wherein the DNA construct further comprises a positive selection marker, and the method further comprises the step of selecting for cells comprising said positive selection marker.

73. The method of claim 72, wherein the DNA construct further comprises a negative selection marker, and the method further comprises the step of selecting against cells comprising said negative selection marker.

74. The method of claim 72, wherein the positive selection marker is the *neo* gene, and cells comprising said positive selection marker are selected by culture in medium comprising G418.

75. The method of claim 73, wherein the negative selection marker is the *gpt* gene, and cells comprising said negative selection marker are selected by culture in medium comprising 6-thioxanthine.

76. The method of claim 73, wherein the negative selection marker is the Herpes Simplex Virus thymidine kinase gene, and cells comprising said negative selection marker are selected by culture in medium comprising gancyclovir.

77. (Amended) A non-immortalized primary or secondary cell produced by the method of claim 65.

78. (Amended) A clonal cell strain of secondary cells produced from a primary or secondary cell of claim 77.